CHAPTER ONE

Combinatorial Chemistry and Multiple Parallel Synthesis

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1 INTRODUCTION

The introduction of a new pharmaceutical is a lengthy and expensive undertaking. Methods which promise to shorten the time or the cost are eagerly taken up, and this is clearly the case with combinatorial chemistry and multiple parallel synthesis.

Combinatorial chemistry is somewhat hard to define precisely, but generally speaking, it is a collection of methods that allow the simultaneous chemical synthesis of large numbers of compounds using a variety of starting materials. The resulting compound library can contain all of the possible chemical structures that can be produced in this manner. Multiple parallel synthesis is a related group of methodologies used to prepare a selected smaller subset of the molecules that could in theory have been prepared. The content of libraries prepared by multiple parallel synthesis is more focused and less diverse than those constructed with combinatorial technology.

The primary benefit that combinatorial and multiple parallel chemistry bring to drug synthesis is speed. As with most other human endeavors, uncontrolled speed may be exhilarating but is not particularly useful. Rapid construction of compounds that have no chance of becoming drugs is of little value to the medicinal chemist. After an initial euphoric period when many investigators thought that any novel compound had a realistic chance of becoming a drug, realism has now returned, and libraries are being constructed that reflect the accumulated wisdom of the field of medicinal chemistry. Combinatorial methods have permeated and irreversibly altered most phases of drug seeking that benefits from the attention of chemists. The successful contemporary medicinal chemist must be aware of the strengths and weaknesses of these exciting new methods and be able to apply them cunningly. In the proper hands combining medicinal chemical insight with enhanced speed of synthesis is very powerful.

Libraries are constructed both in solution and on solid supports and the choice between these techniques is often a matter of personal preference, and they are performed side by side in most laboratories. For very large libraries, however, construction on resins is more practical, whereas for smaller, focused libraries, solution phase chemistry is more practical. Solid phase methods are also specially advantageous for multistep iterative processes and are notable for the comparative ease of purification by simple filtration and the ability to drive reactions to completion by the use of excess reagents. Throughout the previous decade, solid phase organic synthesis (SPOS) has dominated combinatorial chemistry, and many novel methods have been developed as a result.

	A	B	C	D	E
A	AA	BA	CA	DA	EA
B	AB	BB	CB	DB	EB
C	AC	BC*	CC	DC	EC
D	AD	BD	CD	DD	ED
E	AE	BE	CE	DE	EE

Figure 1.1. A combinatorial library constructed from five reacting components.

The main concepts in this field can be summarized in Figs. 1.1 and 1.2. In Fig. 1.1, there is a hypothetical combinatorial compound library of condensation products produced by reacting every possible combination of five starting materials. This results in a library containing 25 (5×5) products. The library could be constructed by 25 individual reactions, with each product separate from all of the others. It could also be constructed by run-

	A	B	C	D	E
C	AC	BC*	CC	DC	EC

Figure 1.2. A multiple parallel synthesis library constructed from six participants.

ning reactions simultaneously so that a single mixture of all 25 substances would be obtained.

In this specific example, it is presumed that the reactions were run in a single step so that all compounds were produced simultaneously in a big mixture. Furthermore, it is assumed that the ideal was achieved. That is, all reactions proceeded quantitatively, and that each compound is present in the final compound collection in equal molar concentration. (This ideal is rarely achieved.) It is also assumed that BC is the only active constituent and so it is marked off with an asterisk. If the whole library were tested as a mixture, then it would be seen that it contained an active component, but one would not know which one it was. For this to happen, it is necessary that the components do not interfere with one another so false positives and false negatives are not seen. Many clever means of finding the active component expeditiously have been developed and a number of these will be illustrated later. It is clear that if the components were prepared and tested individually, 25 separate reactions would be required and the identification problem would disappear, but great strain would be placed on the bioassay and the speed of synthesis would be compromised. A perfect combinatorial library for drug discovery would only contain BC, and only a single reaction would be required, but this level of efficiency is rarely achieved by any contemporary medicinal chemical method. The real problem is to construct a compound library that is sufficiently diverse and sufficiently large that there is a high possibility that at least one component will be active in the chosen test system. This example assumes that this has been done. The reader will also readily see that with the library in Fig. 1.1, that instead of testing all of the compounds simultaneously, if one prepared and tested 10 mixtures resulting from combining the five products in each column and each row, then BC would reliably emerge as the active component because only the row C mixture and the column B mixture would be active, and the active component must be the one where the rows and columns intersect. When more than one active component is present then the problem becomes more complex.

Figure 1.2 illustrates a related multiple parallel library. This is much smaller and starts with the assumption that one knows or suspects that the best compound will terminate in component C. Five reactants are chosen to condense with C and the resultant library consists of five components. Each of these products is tested singly and BC* is quickly identified.

The greater efficiency of this library compared with that of Fig. 1.1 for the purpose is obvious. Clearly, the smaller the library that succeeds in solving the problem, the more effective the process is. A perfectly efficient library would only contain BC*. The size of the library produced by either method is the product of the number of variables introduced and the number of steps involved raised exponentially. For example, starting with a given starting material (often called a centroid) and attaching four different groups of 10 sidechains to each product would produce $1 \times 10 \times 10 \times 10 \times 10$ or 10,000 members. The primary advantage of either method is speed, because the products are prepared simultaneously at each step. The efficiency is also enhanced when the condensation steps involve the same conditions.

The use of the library depends on the specific structures included and the purposes for which the library is to be tested. The relevance of speed to drug discovery is easy to explicate. If one knew in advance the particular structure that would satisfy the perceived need, a successful compound library would only need to have one substance in it. If one has a general idea of the type of structure that would be useful, the library will have many promising compounds but still be finite in number. Quantitative bioassay of pure substances would allow one to select the most nearly perfect embodiment. If one has no idea of the type of structure that would give satisfaction, then a successful library must have a larger and more diverse number of compounds in it.

Contemporary drug seeking is a complex, time consuming, and expensive process because a successful drug must not only have outstanding potency and selectivity, but it must also satisfy an increasingly long list of other structure-dependent criteria as well. The elapsed time from initial synthesis to

marketing is estimated to lie on average between 10 and 15 years and to require the preparation and evaluation of a few thousand analogs. The costs are estimated to lie between $300 and $800 million per agent. Most large firms now target the introduction of 1–3 novel drugs per year and target sales at a billion dollars or more from each. This indicates that each day of delay in the drug seeking process not only deprives patients of the putative benefits of the new drug but also represents the loss of a million dollars or more of sales for the firm! Added to this is the intense competition among big pharmaceutical companies for a winning place in the race and among small pharmaceutical companies for survival. First to market in an unserved therapeutic area can return a great profit if a sufficient number of sufferers exist who have access to the funds to pay for their treatment. The next two entries competing with this agent can also be expected to do well. After this, success is rather more problematic because the market grows more and more fragmented. Being first to finish the race, therefore, conveys very real survival value. From an economic standpoint, it is estimated that less than 10% of products introduced repay their development costs. Those few that do must return a sufficient surplus to amortize the costs of the rest and sufficient additional funds to cover the costs of future projects and to gratify the shareholders. These imperatives have placed a premium on speed of discovery and development. The portion of this time devoted to synthesis and screening in the drug-seeking campaign is usually about 3–5 years. The enhanced speed of construction can be expected to decrease the time to market by perhaps as much as 1 year in favorable cases. While this is less than was originally hoped for when these methods were introduced, it is not trivial.

Combinatorial chemistry is now such a pervasive phenomenon that comprehensive review of its medicinal chemical features is no longer possible in less than book length. Full coverage would require treatment of its impact on all of the phases of drug discovery and would exceed the space available. Thus, the remainder of this chapter will illustrate its main features and applications.

2 HISTORY

Combinatorial chemistry grew out of peptide chemistry and initially served the needs of biochemists and the subset of medicinal chemists who specialized in peptide science. Its first decade or so concentrated on oligopeptides and related molecules. It continued to evolve, however, and now permeates virtually every corner of medicinal chemistry and a major effort is underway to discover new, orally active, pharmaceuticals using these methods.

Many will agree that the path leading to the present state of combinatorial chemistry essentially started with the solid phase synthetic experiments on peptides by Bruce Merrifield in 1962 (1, 2). This work had immediate impact, facilitated in large part because of the essentially iterative reactions, to completion by use of reagents in excess, its susceptibility to automation, and the ease of removing detritus from the products by simple washing and filtration away from the resins. At first this extremely useful technology was employed in a linear fashion. It was probably Furka in Hungary a decade or so later who realized that the methodology could lead to simultaneous synthesis of large collections of peptides and conceived of the mix and split methods (3). Geyson made the whole process technically simpler in 1984 and produced large scale compound collections of peptides (4) and Houghton introduced “tea bag” methodologies in 1985 in which porous bags of resins were suspended in reagents (5). Comparatively few organic chemists undertook the preparation of ordinary organic substances on solid phases because the work is rather more complex when applied to non-oligomeric substances caused by greater variety of reactants and conditions required, and this work at first failed to develop a significant following. Solid phase organic chemistry was also comparatively underdeveloped and this held back the field. This changed in dramatic fashion after the publication of Bunin and Ellman’s seminal work on solid phase organic synthesis (SPOS) of arrays of 1,4-benzodiazepine-2-ones in 1992 (6). Soon other laboratories published related work on this ring system, and work on other drug-like molecules followed in rapid order and the race was on. In the initial phases, solid phase or-

ganic synthesis predominated, and this persisted until about 1995, when solution phase combinatorial chemistry began to make serious inroads. Until about 1997, roughly one-half of the libraries reported were either of peptides or peptidomimetics. Subsequently libraries of drug-like small molecules have become increasingly popular.

The work on combinatorial libraries has inspired the rapid development of a wide variety of auxiliary techniques including the use of reagents on solid support, capture resins, chemical and biological analysis of compound tethered to resins, informatics to deal with the huge volume of structural and biological data generated, the synthesis of a wide variety of peptide-like and heterocyclic systems hitherto prepared solely in solution, photolithographic techniques allowing the production of geographically addressed arrays on a "credit card," preparation of gene array chips, attachment of coding sequences, use of robotics, and the preparation of oligonucleotides by Letsinger in 1975 (7) and of oligosaccharides by Hindsgaul in the 1990s (8). At this moment several thousand papers are appearing each year describing the preparation and properties of compound libraries either in mixtures or as individual substances. Several books (9–35) and reviews (36–49) are available for the interested reader. Those of Dolle are particularly recommended because he has undertaken the heroic task of organizing and summarizing each year the world's literature on the topic. That of Thompson and Ellman is especially thorough in reviewing the literature up until 1996 from a chemical viewpoint. A great many other reviews are available, including many in slick-cover free journals that arrive on our desks weekly. In addition to these, at least three specialist journals have been established in the area. These are the *Journal of Combinatorial Chemistry, Molecular Diversity*, and *Combinatorial Chemistry and High Throughput Screening*.

Another important factor leading to the explosion of interest in combinatorial chemical techniques was the development of small firms devoted to the exploitation of genetic discoveries through development of high-throughput screening methods. These firms by and large did not have libraries of compounds to put through these screens and were seeking collections of molecules. Combinatorial chemistry addressed these needs. When these methods were taken up by big pharmaceutical companies, existing libraries quickly proved inadequate for the need and combinatorial methodologies clearly addressed this need as well. Just about 10 years after these seminal events, the face of medicinal chemistry has been irretrievably altered. While combinatorial chemistry has in some respects not lived up to the initial hopes, its value is firmly established and no serious firm today fails to use these methods. By the year 2002, well over 1000 libraries have been reported. Many of these include reports of the biological activity of their contents. This is remarkable considering that the field is scarcely more than a decade old!

3 SOLID PHASE ORGANIC SYNTHESIS OF INFORMATIONAL MACROMOLECULES OF INTEREST TO MEDICINAL CHEMISTS

That the solid phase synthesis of collections of peptides launched this field is not intrinsically surprising. The basic methodology existed because of Merrifield and many others. The peptide linkage has notable advantages for this work because it is relatively chemically stable, non-chiral, constructible by iterative processes amenable to automation, the products are rarely branched, possess a variety of interesting biological properties, and can be constructed in great variety. The counterbalancing defects of these compounds are that they are not easily delivered orally unless they are end capped and rather small in molecular weight, are readily destroyed by enzymatic action, and fail to penetrate into cells. The physiological reason for this is readily understood. Peptides, and other informational macromolecules, function in the body to provide specific structure or to generate signals for cells to respond to according to their sequence and architecture. It would be dangerous if they were absorbed intact from ingestion of other life forms. To prevent cellular disruption they are first digested in the gastrointestinal tract, absorbed as monomers, and then reassembled after our own genetic pattern so that they join

or supplement those already present without causing disruption in cellular architecture or function.

Nucleic acids have many of the same advantages and disadvantages, and their compound libraries came into being soon after the peptides. Oligosaccharides, on the other hand, have lagged considerably behind. They possess chiral linkages whose construction must be carefully controlled, often are branched, are fragile in the presence of acid conditions, are often highly polar, and are readily digested. Controlling these processes is much more difficult and it has taken longer to conquer these problems.

3.1 Peptide Arrays

Peptides are prominent among the compounds of interest to biochemists but less so to most medicinal chemists for reasons explicated above. Construction of peptides of specified sequence is an iterative process whose complications largely consist of protection-deprotection steps to ensure that side-chain functionality does not interfere with orderly amide bond formation. When made on resin beads, there are limits to the quantities that can be made in part because of the geometric restrictions caused by bead size and the need to avoid adjacent molecules from interacting with each other, and the comparative lability of the beads to aggressive reagents places limitations on the chemical conditions that can be employed. Porous beads obviously can be loaded more heavily than those whose surface only is accessible after wetting by the reagents. Many different kinds of resins and other solid supports are now available, including some that are solvent soluble depending on the solvent and the temperature.

With their balance of advantages and disadvantages and the present gold standard being oral activity, peptide libraries are currently of primary value in lead seeking, in basic studies on cellular processes, or for the preparation of parenteral medications. Despite intensive study spanning several decades by some of the best minds of this generation, means of delivering thereputically significant blood levels of peptides through the oral route remain elusive. Translating the therapeutic message in peptide leads into oral non-peptide drugs through generally applicable systematic techniques has also been elusive. The several successes that have been achieved have been primarily the result of screening campaigns or serendipitous observations, and the results have so far not revealed an underlying tactical commonality that can be exploited in new cases. Perhaps the best known of these studies has been the translation of snake venom peptides, whose injection by serpents leads to a precipitous fall in blood pressure, into truncated pseudodipeptides like captopril, and then on to enalaprilat and lysinopril, which are pseudotripeptides. The basic lesson learned from all of these studies has been that the resulting agent should be as little peptide-like as possible and not exceed the equivalent of at most four amino acid-like residues. Examination of peptide structures in light of the well-known Lipinski rules provides a rationale for what experience has shown. Beyond about four residues, the molecular weight is becoming too high, the polarity is weighted too much toward water solubility, and the hydrogen-bonding inventory is excessive. Further, the compounds are excessively water soluble so that they do not pass through cellular membranes efficiently by passive diffusion.

An added feature to bear in mind is that the preparation of certain medically important polypeptide drugs, such as human insulin and growth hormone, through genetic engineering methodologies, is well developed and convenient so these substances can be used in parenteral replacement therapy. Their preparation through synthetic peptide chemistry represents important achievements in peptide intellectual technology but does not satisfy a commercial need.

Nonetheless, peptide compound libraries are very convenient for uncovering leads quickly for receptors where natural ligands or serendipitous drugs have not previously been found and large libraries of peptides continue to be made (50–65).

The number of peptides that could in principle be made is stupefying. For example, given that there are approximately 20 common amino acids, and allowing five post-translational modifications (and ignoring the fact that there are more of these and that there are many wholly synthetic amino acids), the avail-

Table 1.1. Number of Possible Peptide Products as a Function of the Amino Acids Used

Dipeptides	(20×20)	$= 400$
Tripeptides	$(20 \times 20 \times 20)$	$= 8000$
Tetrapeptides	$(20 \times 20 \times 20 \times 20)$	$= 160{,}000$
Pentapeptides	$(20 \times 20 \times 20 \times 20 \times 20)$	$= 3{,}200{,}000$
Hexapeptides	$(20 \times 20 \times 20 \times 20 \times 20 \times 20)$	$= 64{,}000{,}000$
Heptapeptides	$(20 \times 20 \times 20 \times 20 \times 20 \times 20 \times 20)$	$= 1{,}380{,}000{,}000$

able synthons are at least approximately equal to the number of letters in the Western alphabet. By analogy with the number of languages that have been generated using this system, the potential number of peptides that could be made is clearly astronomical. It would require an incredible effort to make a library containing even only one molecule of each, and Furka has estimated that the mass of such a library would exceed that of the universe by more than 200 orders of magnitude (3)!

Were one to use just the common amino acids, the progression of peptides possible is enormous, as is shown in Table 1.1.

The simplest and least ambiguous method for constructing peptide libraries is the spatially separate or spatially addressed method. Here a single peptide is constructed on a single type of resin, and the resin/products are kept separate. No decoding sequence needs to be attached to the beads in this kind of library. This method was introduced by Geysen in 1984. To make 96 peptides at a time and to keep track of the products and facilitate their screening, the reactions were run on resins attached to individual pins so constructed that they fit into individual wells of 96-well plates. (Fig. 1.3) (66). A convenient variation was developed for parallel synthesis in which beads were contained in porous bags and dipped into reagent solutions. These are called "tea bags." The identity of the peptide or peptides contained is recorded on the attached label. Subsequently Fodor et al. developed a very diverse library on silicon wafers using photolithographic chemistry for forming the peptides and controlled the specific place along an *x–y* axis, where each peptide would be located through use of variously configured masks (Fig. 1.4.) Photolytic protecting groups were employed followed by coupling the newly revealed "hot spots" with a suitable reactant. After this, the masks are moved as often as desired and the process repeated. In principle this method could produce thousands of individual peptides on a credit card–like surface. Although somewhat laborious, the synthesis can readily be automated. The method requires photosensitive protecting groups and testing methodologies compatible with support-bound assay methods and the libraries are geographically coded by the position of products on the *x–y* axis (67). These techniques are now widely employed for gene array amplification and identification experiments.

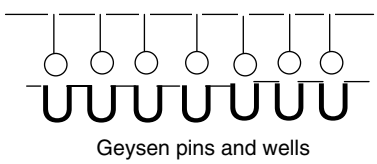


Figure 1.3. Geysen pins and wells.

Synthesis of mixtures of peptides further enhanced the speed and convenience of library construction but required development of devolution methods so that active components in the mixtures could be identified. Direct methods of sequence analysis are available. Mass spectrometry is popular as are NMR methods (involving magic angle methods on single beads). Edman degradation of peptides can also be performed. These methods are popular when iterative methods result in linear polymers.

A further complication of simultaneous preparation of peptide mixtures is that individual amino acids differ greatly in their reactivity, so if one simply placed all of the potential reactants in a flask under bond forming conditions, they would not react at the same rate. With each iteration, the disparity between readily formed and poorly formed bonds would widen. One way to deal with this problem is to use less than fully equivalent

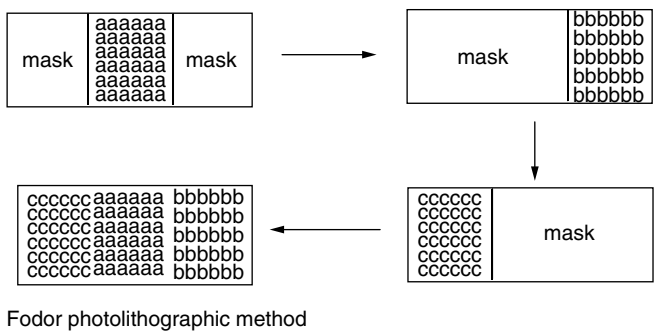


Figure 1.4. Fodor photolithographic method.

amounts of each component and to allow the reactions to go to completion. For example, if two components are employed, each should be added in about one-half molar quantities. At the end of the reaction, one should have equimolar amounts of both products. This would allow the subsequent testing results to be quantitatively comparable (68). Alternatively, split synthesis methods were developed contemporaneously by Houghton (69), Furka (70), and Lam (71). In split and pool synthesis (Fig. 1.5), an initial reaction is run on a bead support to attach an amino acid as before, and the resulting beads are then split into equal portions and each of these groups of beads is deprotected and reacted with one of a group of different second amino acids to form dipeptides. These are pooled again and thoroughly mixed. This pool is again separated into equal portions and each is reacted with one of a different group of other amino acids to produce a group of tripeptide mixtures. This is continued until satisfied, and the last group of resin piles is usually not mixed. Although each individual bead will contain a single peptide, the final products from this methodology consist of groups of related materials. By illustration, if run in the manner described, one could start with the same amino acid attached to a bead. If this resulted in 20 piles of beads, each with a different amino acid attached, then each pile could be reacted in parallel subpiles with a different one of the 20 amino acids and repooled. Continuation would lead to 20 collections of all of the possible tripeptides. Detaching and testing would reveal which was the best amino acid to start with (XXB in the example shown in Fig. 1.6). Keeping this one constant in the next series, repeating the process with the remaining 19 would reveal the best second amino acid (XEB in the example). Iteration would lead, after significant labor, to the optimal sequence at all positions and produce a structure-activity relationship based on those other substances approaching its potency (GEB $>$ IEB $>$ HEB in the example). This method of deconvolution is known as positional scanning.

A great diversity of peptides can be constructed in short order using these methods, and very large libraries have been produced. Such collections would be truly combinatorial. Many subvariations of this process can be envisioned. In general it is found that more than one active peptide is obtained. Synthesis of all of these actives as individual pure chemicals (often called "discretes") will allow the development of a structure-activity relationship. No assumptions are made in pursuing the study in this manner. If one has, however, a lead peptide already or wishes to define a re-

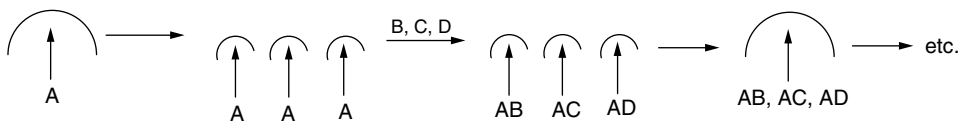


Figure 1.5. Pool and split method.

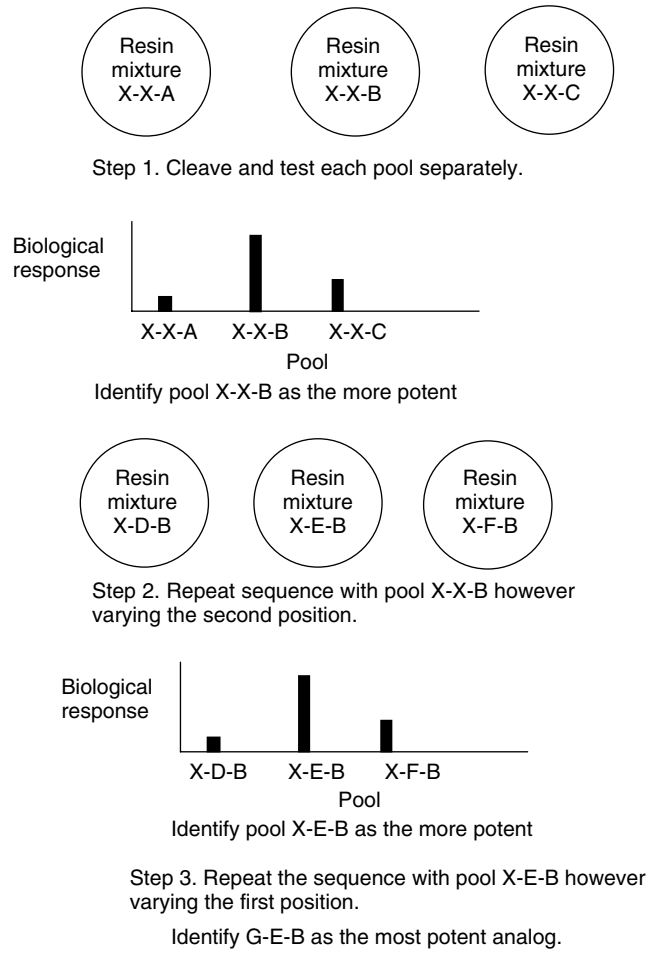


Figure 1.6. Split and mix method with positional scanning.

gion in a lead peptide, then one can perform a more limited study by systematically varying all of the individual positions in the region in question in this manner. The general experience is that potency and selectivity increases as each position is optimized and the known sequence grows (72). Although this method is somewhat wasteful, it has no preconceptions, and the residual sequences not of interest in this test series can be archived and examined in future test systems. Another advantage of the method is that the final products are not tethered so are able to assume the solution conformation dictated by their sequence or by the receptor interaction and also to interact with insoluble receptors.

In the substitution/omission method (Fig. 1.7), one starts with a lead sequence whose activity is known and replaces sequentially each amino acid with all possible 20 analogs, keeping the remainder the same. This is similar to the divide-couple-recombine method just described, but it evaluates only a single specific residue at a time. Testing the 20 libraries reveals which amino acid is optimal at that position. This is repeated until all of the amino acids in the sequence being examined have been evaluated and an optimized sequence is at hand. In the illustration, one starts with known sequence A-B-C-D and discovers enhanced potency in the modified sequence of E-F-C-D.

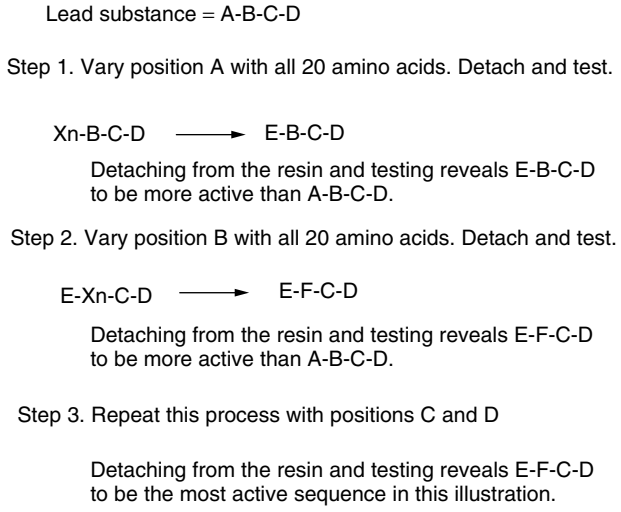


Figure 1.7. Substitution/omission method.

In the omission method, one deletes one of the amino acids from a given position (most often at the end) or replaces an amino acid residue at any chosen position by an alanine or glycine and finds which omission decreases activity. This is done at each position until the optimal sequence is detected and the relative contribution of each amino acid side-chain is clear.

Devolution by positional scanning is facilitated through testing groups of resins arranged in checkerboard rows and columns as illustrated earlier in Fig. 1.1. Subsequent libraries are much smaller so the process becomes progressively less laborious.

Another popular method of identifying residues is to attach a non-peptide signaling molecule orthogonally to a different attachment arm whose reactivity differs from the first each time an amino acid is attached to its arm (Fig. 1.8). The signaling or coding molecule needs to be attached to its arm using chemistry that does not interfere with the growing peptide on the other arm and does not detach either molecule prematurely. There are a variety of strategies employed in detaching the sequences from the arms (Fig. 1.9). Strategy a represents an internal displacement reaction and leaves no trace behind in the product of the original point of attachment. Strategy b uses an external nucleophile for attachment. This completes the product structure and usually does not leave a linker trace. Strategy c is a reductive or hydrolytic strategy and sometimes leaves a linker trace in the product but need not do so. The signal sequence carries the history of the bead and therefore codes for the history of the steps involved in the synthesis and thus for the identity of the peptide that one believes one has attached to the bead. The ease of identification of oligonucleotide sequences (by PCR methods) has made these popular for such coding. Various halogenated aromatic residues have also been used for this purpose. Another popular method is to embed an rf generator tuned to individual frequencies in the resin itself so that the substance on the bead can be identified by tuning to the proper frequency. One can also place bar codes on the beads for convenient reading. Much ingenuity has been expended on ingenious methods of deconvolution.

The development of these methods presents the medicinal chemist with the ability to perform a chemical evolution. This would seem to parallel nature's use of biological evolution to produce chemical libraries suitable for particular biological purposes. Chemical evolution of this type is more congenial to the impatient chemist.

Clearly with very large libraries, it is technically not possible to analyze each product, so

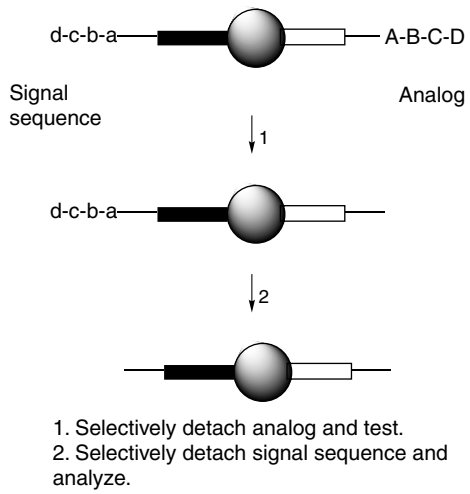


Figure 1.8. Resin with arms containing an analog and a signal sequence.

one must resort to statistical sampling instead or take it as an article of faith that each compound has been successfully prepared. Much work has been devoted to dealing with this problem but a comprehensive treatment of this complex topic is unfortunately too vast to cover here. Clearly careful rehearsal and fanatic attention to detail in the construction of the library helps, but this is conjecture not science. There are relatively few instances in the literature where a careful census has been performed from which to form an opinion on this topic. In one important recent example, a statistical sampling of 7.5% of the contents of a library of 25,200 statin-containing pseudopeptides showed that 85% had the anticipated structure. This is reasonably good for such complex work, but leaves one with a sense of unease in that about 3800 wrong substances were available. It is important also to note that the wrong structures were not statistically distributed among all of the targeted compounds but rather showed a bias toward certain structures. This is not particularly surprising but underscores the importance of the topic when interpreting the results of screening (73).

Another method of devolution uses a method descended from early work of Pasteur for this work. Here, the resins are poured onto a solid surface previously seeded with a microorganism lawn or a substrate that generates a color. Those resins that contain an active material give a zone of inhibition or a color response. Both of these endpoints can be detected visually and the active resins taken off of the surface with tweezers. The active component can then be detached for analysis and identification.

Initially, combinatorial libraries of peptides consisted primarily of products made from linear combinations of naturally occurring amino acids. Subsequently just about every devise employed in ordinary peptide work has been applied to combinatorial studies, leading to a persistent evolutionary drift away from collections of natural peptides. For example, to enhance the metabolic stability of such libraries, end capping of the amino end and the carboxy end and also cyclization have been employed to stabilize these substances. Before long, the incorporation of unusual amino acids also began (Fig. 1.10). These substances can be termed pseudopeptides

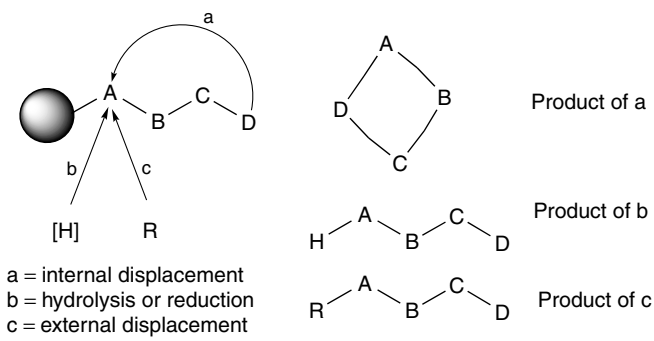


Figure 1.9. Some detachment strategies for detachment of compounds from resins.

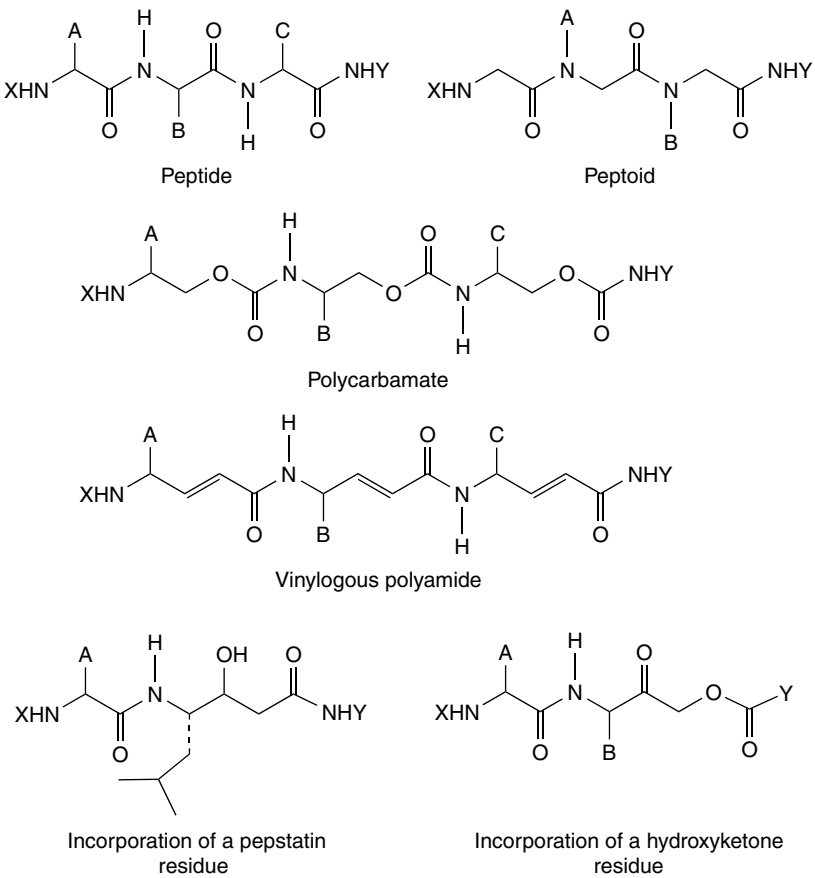


Figure 1.10. Some peptide surrogates employed in libraries.

(74–101). More specifically, such residues as benzamidines (102, 103), phosphinates (104–106), methyleneketones, hydroxyketones, fluoroamides (107), ketoamides, hydroxamates (108, 109), glycols, coumarins (110), boronates, oxazoles (111), nitriles, aldehydes, halogenated ketone hydrates, sulfonamides, and the like progressively appeared. These unusual residues were predominantly incorporated either at the end or at a point where the natural peptides would be cleaved by enzymatic action. These moieties are of special value when the substitution takes place at a site where the processing enzyme must act and employs mechanism-based inhibitory mechanisms. More recent libraries have appeared in which the overall conformation of the peptide has been mimicked so that the resulting heterocycle resembles topographically a β-turn, for example, but may not incorporate any common amino acid components (112, 113). The figure illustrates some of the groups so employed.

Later, the peptide linkage itself began to be modified (Fig. 1.11). For example, the classical mode of stabilization against peptidase cleavage by conversion of the peptide bond NH into *N*-methyl subsequently evolved into the preparation of peptoids (polyglycine chains with each NH replaced by a variety of *N*-alkyl groups of the type that resemble the sidechains found in normal amino acids). Libraries of these compounds were very popular for a while but interest has decreased as time passes (114, 115).

Flirtation with other substitutes for normal peptide bonds includes preparation of libraries of polycarbamates (116), vinylogous amides, incorporation of a pepstatin residue

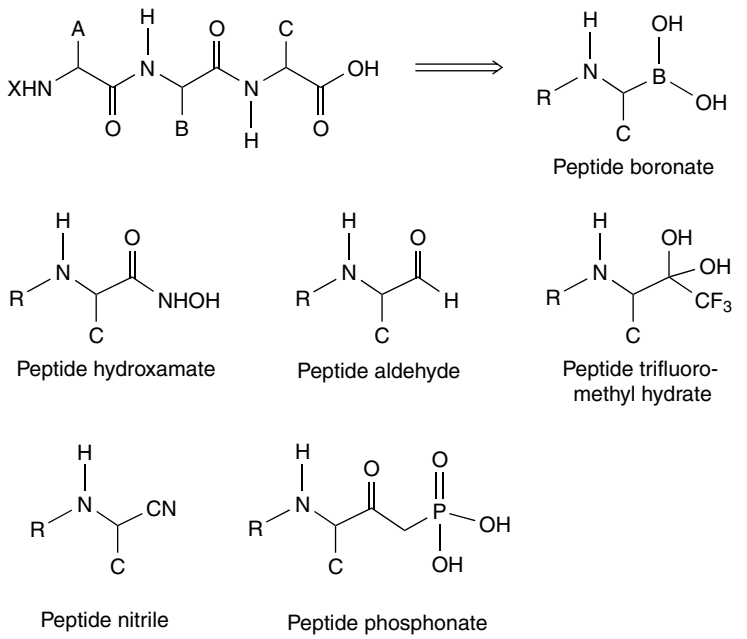


Figure 1.11. Peptides substituted with unusual carboxyl surrogate residues.

(117), and ureas (118) as well. In these libraries, the side-chains project from each fourth rather than each third atom in the chains so these are not close models of amino acids. Such libraries, not surprisingly, are more often antagonist rather than agonist.

In Fig. 1.11, one sees the insertion of unusual peptide bond surrogates for lead seeking. Such residues include peptide boronates, peptide hydroxamates, peptide aldehydes, peptide trifluoromethylketone hydrates, peptide nitriles, peptide phosphonates, and so on. One could also add to this list inclusion of β-turn mimics, β-sheet analogs, and so on. This is at present a very active subfield of medicinal chemistry.

These peptide and pseudopeptide libraries have been replaced progressively by collections containing smaller and more drug-like molecules. These will be covered in their own section below.

3.2 Nucleoside Arrays

The minimum fully realized library of natural peptides would consist of 20^{20} components. An analogous library of nucleic acids would consist of 5^5 components (double this if one used both ribose and deoxyribose units) and be substantially smaller (Fig. 1.12a). Once again, the use of post-translationally modified bases or wholly unnatural analogs increases the attainable diversity. Conformational effects and self-associations further enhance the diversity. Once again, construction is iterative and bead-based automated procedures are available. Libraries of significant size have been constructed and evaluated (119).

3.3 Oligosaccharide Arrays

Construction of diverse oligosaccharide libraries is much more difficult. The linkage is chiral and relatively hard to control, the bonds are acid fragile, and there are many potentially competing functional groups that can be points of attachment (Fig. 1.12b). Despite these complicating factors, such libraries are beginning to appear. Clearly progress is being made.

3.4 Lipid Arrays

Lipid libraries have largely been neglected. For saturated fatty acids, the construction of carbon–carbon bonds is more difficult. Ste-

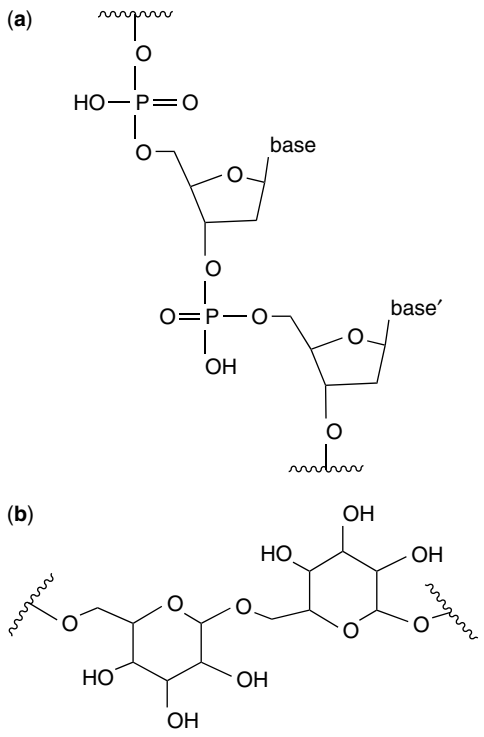


Figure 1.12. (a) Oligonucleotide libraries. (b) Oligosaccharide libraries.

roids and other polyisoprenoids are also complex to construct. Mixed triglycerides would seem accessible, and one anticipates developments in this area.

4 SOLID AND SOLUTION PHASE LIBRARIES OF SMALL, DRUGABLE MOLECULES

As noted above, the field of combinatorial chemistry and multiple parallel synthesis started with libraries of peptides. In time, unusual residues crept into the products. While this evolution is still ongoing, it is now accompanied by a major effort to produce libraries of small, drug-like molecules in library form. Many of the methods used for large molecules carry over but the largely non-iterative nature of small molecule synthesis is a significant complication.

The current "gold standard" in small molecule drug seeking is oral activity accompanied by one-a-day dosing. This is a high hurdle. The majority of molecules that have passed have molecular weights of about 500 or so. It has been calculated that the number of small molecules that would fall into this category is approximately 10 raised to the 62nd power! Clearly preparing all of these in reasonable time is beyond the capacity of the entire population of the earth even if they worked tirelessly. The number of compounds that can become satisfactory drugs encompassed in this impossible collection is probably in the range of a few thousand, so most of the effort would be wasted. Hence medicinal chemical skills are still at a premium.

Obviously construction one at a time in the usual iterative or non-iterative fashion results in single molecules of a defined nature. Combination of reactants A and B produces a single product A-B. Reaction of this with another substance produces product A-B-C. In each case, a single reaction produces a single product. The change brought about by combinatorial or multiple parallel synthesis methods is that the reactants are usually linear, but the products can be logarithmic. For example, reacting A with 10 different Bs produces 10 products ($A\text{-}B_{1-10}$), and the reaction with 10 different Cs on each of these results in 100 products ($A\text{-}B_{1-10}C_{1-10}$), either in mixtures or as discrete compounds. Rather large compound collections can be assembled quickly using this scheme.

With non-linear products a different variant is seen than experienced with large molecules (Fig. 1.13). Here a starting material (often called a centroid) with a number of functional groups (preferably with different degrees of reactivity—known as orthogonality) can be reacted with a variety of substituents (often called adornments) to produce a large number of analogs. In the figure, one sees illustrated centroids with two, three, or four such functional groups and given the number of possible variants, this can lead to a very large library of analogs in a brief time (two functional groups with 10 variant adornments quickly results in 100 analogs, three in 1000, and four in 10,000). If the reaction conditions allow, these variations can be run in mixture or in parallel effecting a very significant time savings. It can, however, put a sig-

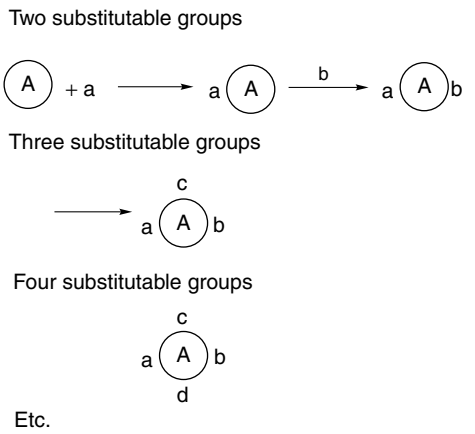


Figure 1.13. Centroid adornment.

nificant strain on purification, analysis, record keeping, budgets, etc. Much work has been expended in addressing these potential limitations.

With 500 as the normal practical upper molecular weight limit, it can be seen that centroid A should be chosen to have the smallest practical molecular weight. It is also helpful if, when fully adorned, it has functional groups remaining that can interact productively with a receptor so the weight devoted to this part of the molecule is not net loss. The molecular weight of the centroid places practical limits on the net weight that the adornments can collectively have. If one adornment is rather large, then this requires one or more of the other adornments to be made compensatingly smaller. The more functional groups present in the centroid, the smaller each adornment can be.

It is particularly helpful if the adornments project into space into quadrants that fit precisely the needs of the receptor if one is optimizing a lead. Alternately, if one is hit seeking, they should project into various quadrants about the centroid so as to allow a fruitful exploration of potential receptor needs. In hit seeking, one often wants molecular flexibility, whereas in lead optimization progressive rigidification is often more effective.

In addition, the adornments must have the usual medicinal chemical characteristics. They should not be chemically reactive, convey toxicity, or be inordinately polar. The product should fit the modified Lipinski rules to allow for the usual molecular weight and lipophilicity creep that often accompanies analoging. The net hydrogen bonding inventories, log P, water solubility, and cell penetrability features set constraints on the individual and the collective nature of the adornments. If one is rather polar, for example, the polarity of another usually must be decreased. Whether the centroid should be tethered to a solid support or free in solution must be considered carefully. If tethered, it is important to consider whether the point of attachment will remain in the final analog, and if so, what affect this may have on its biological properties. One also should consider whether this attachment will prevent the use of one of the potential adornment points.

Just as in one at a time synthesis, linear syntheses are the most risky and produce the lowest yields. Converging methodologies address these limitations successfully, and in combinatorial work, Ugi (four-component) and Passerini (three-component) reactions are very flexible and popular. Generally one has less control over the specific products being produced by such reactions but this is largely compensated for by the molecular diversity available in this way.

Clearly a great deal of thought should go into library design before the work begins.

The first libraries containing heterocycles recognizable as orally active drugs were the 1,4-benzodiazepine-2-ones prepared on resins by Bunin and Ellman in 1992 (Fig. 1.14) (6). A notable chemical feature is the use of amino acid fluorides to drive the amide formation to completion. The choice of benzodiazepines was inspired because of the medicinal importance of these materials and their resemblance to peptides. Here the library was constructed by a combination of three reactants. If each were represented by 10 variations, the library could easily reach 1000 members ($10 \times 10 \times 10$) in short order. This would fit the commonly accepted meaning of combinatorial in that all of the possible variants would be constructed. Being selective in the variations actually incorporated, a smaller ("focused") library could be made that answered specific pharmacological questions but at the risk of missing an unexpected discovery. Such a li-

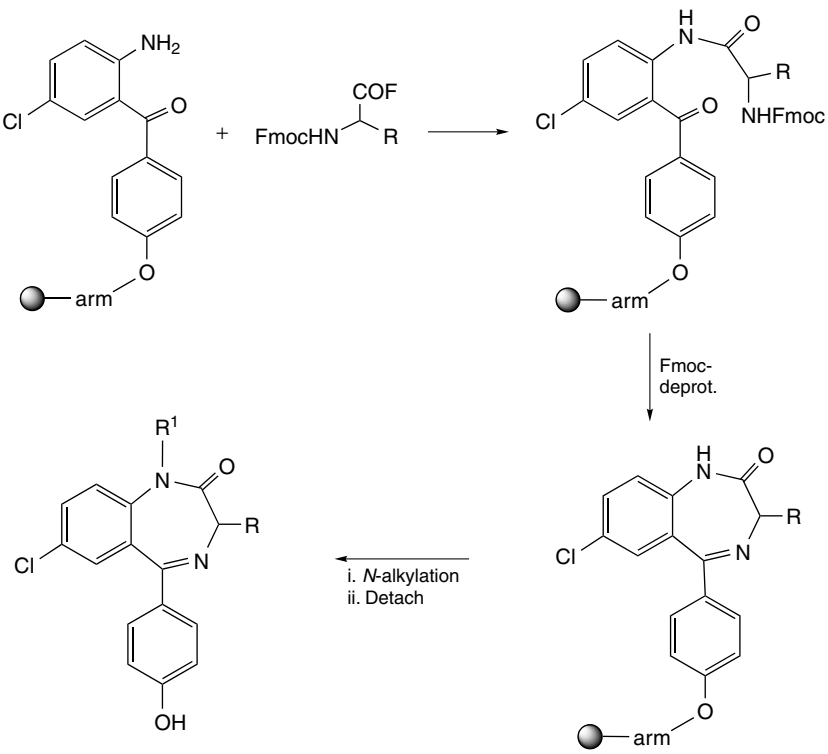


Figure 1.14. Synthesis of benzodiazepine libraries—1.

brary would fit the commonly accepted definition of multiple parallel synthesis. Such focused libraries are often more intellectually satisfying to the practitioner. In this library the attached groups project into space at widely separated compass points around the molecule allowing a systematic exploration of receptor requirements. The centroid has a molecular weight of 160 when all of the available substitution points are occupied by hydrogen atoms. If one accepts an upper molecular weight limit of approximately 500, then four variations can occupy 340 atomic mass units (500–160) so each adornment can have an average of about 85 amu, if the weight is evenly distributed. This gives significant latitude for substitution. When chosen with care to convey drug-like properties and not to exceed collectively the guidelines that Lipinski has developed, the library can contain primarily substances that have a chance to be drug. They also can be so chosen that they allow for substitution independently of each other and also to be installed without premature separation from the beads. It will also be noted that the centroid chosen has amide and amine linkages that are not involved in adornment attachment and are capable in principle of interacting successfully with a receptor so the molecular weight sacrificed to the centroid may perform pharmacodynamic work. Centroids derived from molecular series that are known to be associated with good pharmacokinetics are often referred to as privileged molecules. Thus, the choice of benzodiazepines to demonstrate the potential power of combinatorial chemistry and multiple parallel synthesis was inspired.

In this pioneering library, the final products were attached to the bead support through a phenolic hydroxyl group that remained as such in the products before testing. Varying the point of attachment of the hydroxyl group would lead to additional multiple

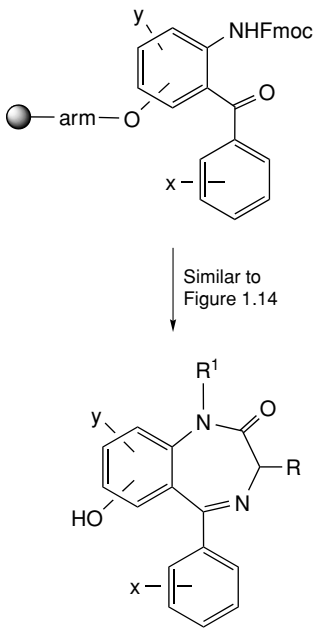


Figure 1.15. Synthesis of benzodiazepine libraries—2.

analogs. Indeed, a variant of this process resulted in a traceable linker in the other aromatic ring (Fig. 1.15).

A somewhat more versatile synthesis of this type using stannanes and palladium acylations (Stille coupling) appeared subsequently (Fig. 1.16) (120). While precedent establishing, this was pharmacologically less than completely satisfying because agents intended to penetrate well into the CNS should not usually contain such a polar substituent. Despite this, several components in these libraries were bioactive, and the work drew widespread attention to the promise of the methodology and was soon followed by a flood of applications to the preparation of drug-like molecules. In this sequence, attachment to the resin was by an amino acid ester bond. Subsequently this bound intermediate was converted to an imine that cyclized to the benzodiazepine moiety on acid cleavage from the resin (Fig. 1.18) (121). The "traceless linker" technology so introduced has now become standard. One of the additional advantages of this application is that incomplete reaction occurring during the synthesis would lead to products that would not cleave from the resin and could be removed by simple filtration. Furthermore, the products were now indistinguishable from benzodiazepines prepared by usual speed analoging (USA) methods.

Ellman's group also developed a traceless linker sequence of a different type based on HF release of an aryl silicon link to the resin (Fig. 1.17) (122).

As it happens, somewhat gratifyingly, testing of these agents revealed no structural surprises. The intense study of the benzodiazepines in the empirical earlier years had apparently not missed much of significance. Nonetheless, these studies resulted in con-

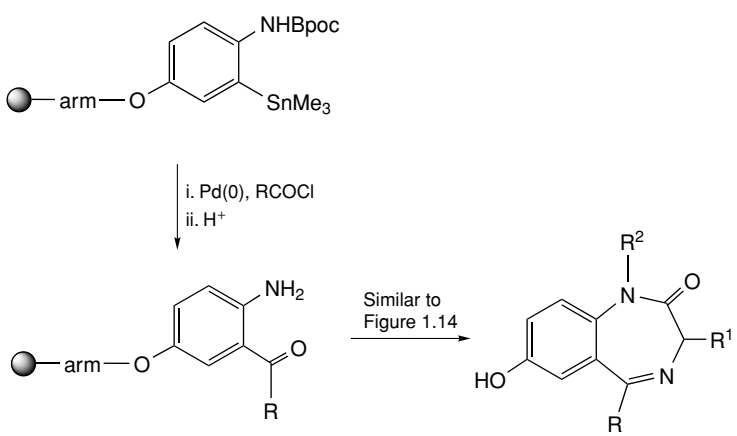


Figure 1.16. Synthesis of benzodiazepine libraries—3.

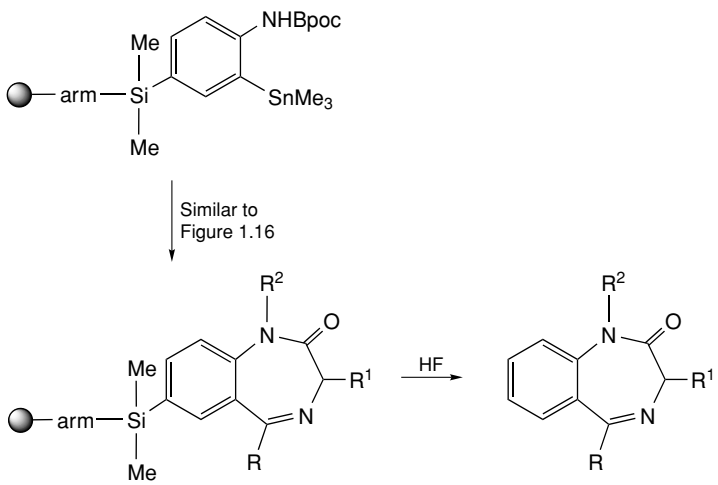


Figure 1.17. Synthesis of benzodiazepine libraries—4.

vincing proof that combinatorial chemical methods would be of dramatic use in preparing agents likely to become orally active drugs.

Benzodiazepine libraries (123) and close analogs such as 1,4-benzodiazepin-2,5-diones (124, 125) continue to be popular. For example, one such library is prepared by a sequence involving a Borch reduction and an internal ester-amide exchange to form the seven-membered ring and then cleavage from the resin. The reaction conditions are mild enough to preserve the optical activity in this library (Fig. 1.19) (126). Use of peptoid starting materials and reductive traceless linker technology are features of the work of Zuckermann et al. (Fig. 1.20) (125).

Representative of another important class of drugs is the 1,4-dihydropyridines. Hantzsch methodology (without the oxidative step) works efficiently on resins for this purpose. The conditions are mild in this synthesis and the yields are good (Fig. 1.21) (127).

Angiotensin-converting enzyme inhibitors are million dollar molecules. An interesting library of captopril analogs were prepared on resin using a split-mix iterative resynthesis deconvolution procedure. From a collection of about 500 analogs, an analog emerged that was threefold more potent than captopril itself and possessed a K_i of 160 p*M* (Fig. 1.22) (128)!

Another important class of contemporary drugs that have been made in library form, in this case both in solution and in solid state, are the fluoroquinolone antimicrobial agents (Fig. 1.23) (129, 130). The solution-based yields were superior to those obtained on the resins.

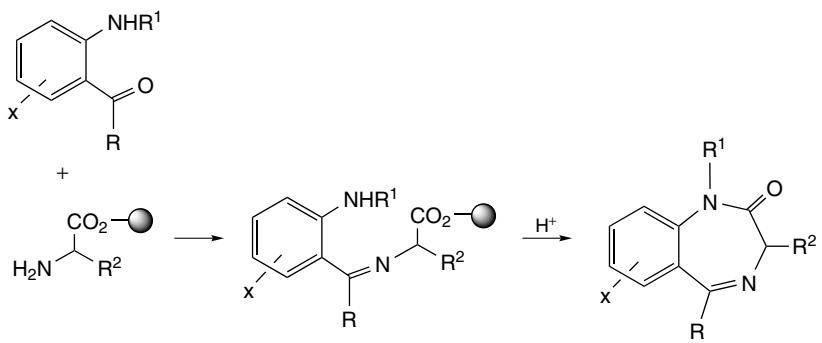


Figure 1.18. Synthesis of benzodiazepine libraries—5.

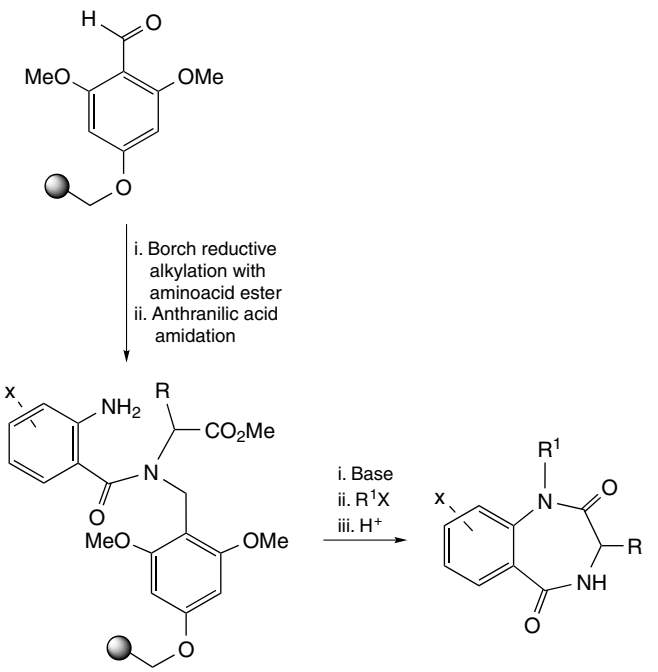


Figure 1.19. Synthesis of benzodiazepine libraries—6.

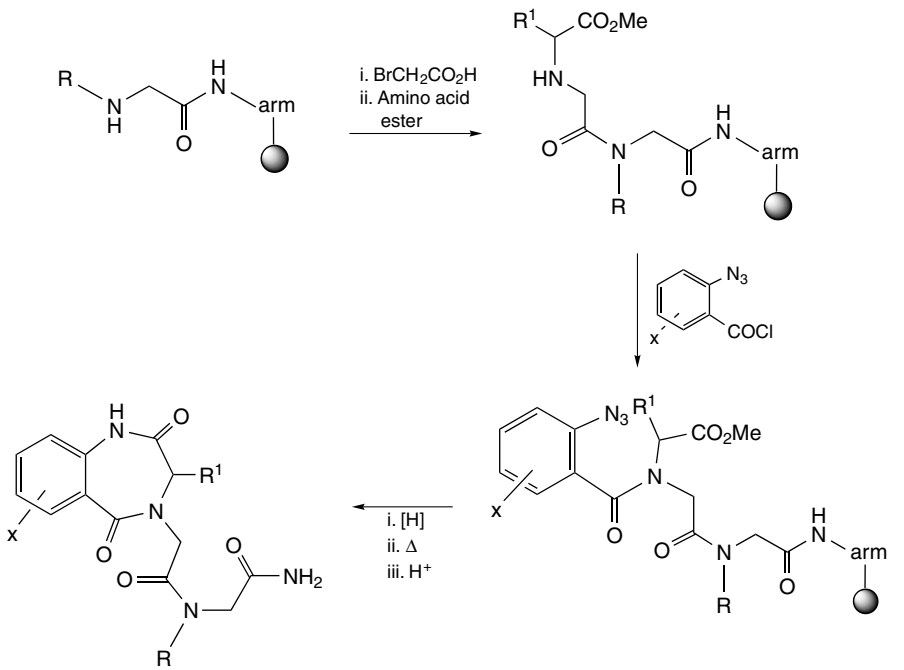


Figure 1.20. Synthesis of benzodiazepine libraries—7.

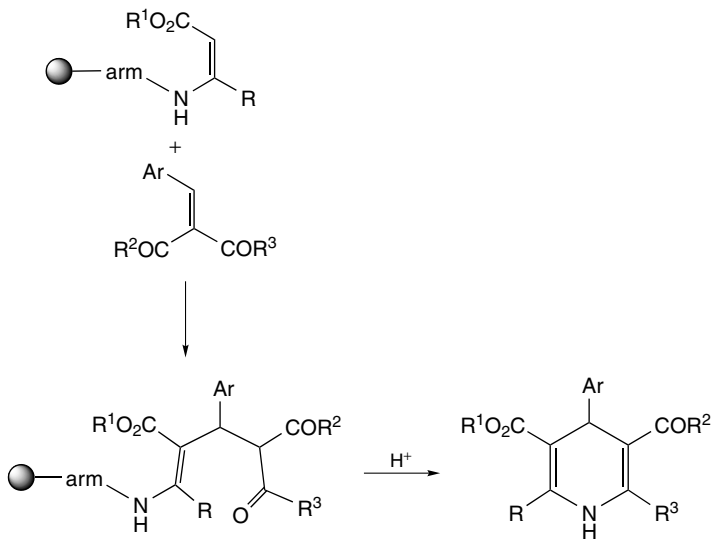


Figure 1.21. Synthesis of dihydropyridine libraries.

Many more examples could be covered, but this gives a representative flavor of the field. By now most of the heterocyclic ring systems have been produced in library form. Particular emphasis has been placed on libraries of molecules with interesting pharmacological properties. Rather extensive reviews of this work exist for the interested reader to consult. [The reviews of Dolle (36–40) and of Thompson and Ellman (41) are particularly useful in this context.]

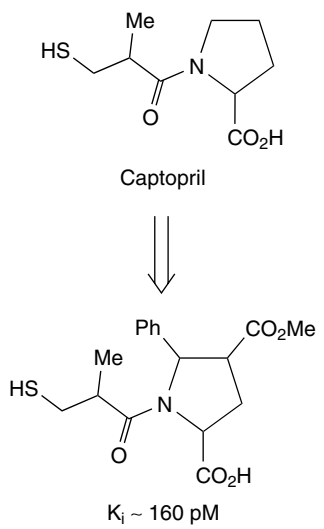


Figure 1.22. Captopril library results.

Recently, focused libraries explore the SAR properties of series of contemporary interest where no drugs have yet emerged or where the first of a promising series has been marketed. An example of this is the oxazolidinones. One example of this class, linezolid, has recently been marketed as an orally effective anti-infective, and most large firms have extensive analog programs in progress in attempts to improve on its properties (Fig. 1.24). Combinatorial chemistry plays a significant role in this work. A Pharmacia group has shown that alteration of the morpholine function to a methylated pyrazole moiety produces a broad spectrum analog with oral activity. Palladium coupling of the iodoaromatic moiety of the starting material allows construction of the trimethylsilylacetylene side-chain. Hydrolysis of this group with formic acid produces a methyl ketone, which after a mixed aldol reaction to the dimethylenamine, reacts with methylhydrazine to produce a mixture of the two possible methylated pyrazole isomers. These are separated by chromatography to produce the best of a large series of analogs produced by these and other reaction se-

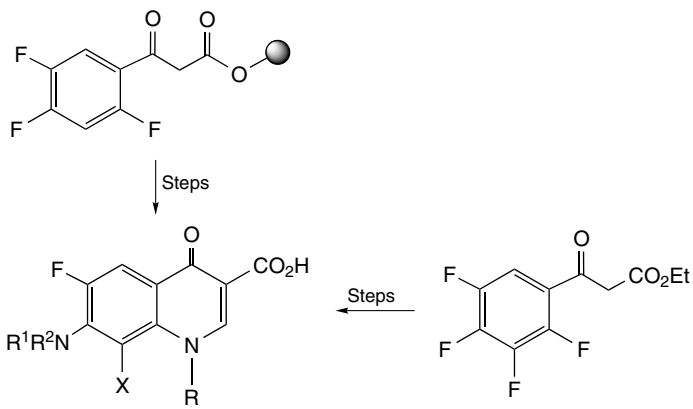


Figure 1.23. Fluoroquinolone libraries.

quences. The product illustrated in Fig. 1.24 has the best combination of *in vitro* and *in vivo* properties in this grouping (131). Reports of related studies have also appeared (132, 133).

A library of cephalosporin antibiotic analogs was made on a solid support (basic alumina) without requiring protection-deprotection. The compounds were prepared in high yield (82–93%) and purity in about 2 min with the aid of microwave irradiation (Fig. 1.25) (134). Microwave acceleration in combinatorial chemistry is a powerful technology for enhancing reactions on solid phases.

The small molecule libraries just exemplified belong to the class called focused. That is, in each case a discrete molecular target was available at the outset and chemical routes were generally available. After some adaptation to the needs of the method and rehearsal of the chemistry, libraries could be generated relatively quickly. Many analogs were then available by comparatively simple variations in the reactants employed. Clearly, in drug seeking, one can operate in much the same manner after identification of a suitable hit molecule.

The strategy required in hit seeking, however, is rather different. Here the initial libraries are usually bigger and more diverse. After the library is screened, and useful molecules are uncovered, subsequent refining libraries are employed that are progressively smaller and more focused. Each succeeding library benefits from the information gained in the previous work so this can be considered the chemist's equivalent of biological evolution. As the work progresses, the needs for quantities of material for evaluation become more and more so the work usually proceeds back into the larger scale one at a time mode resembling the BC (before combichem) era.

A couple of examples represent the very large amount of work carried out in this manner. First, consider the discovery and progression of OC 144–093, an orally active modulator of P-glycoprotein–mediated multiple drug resistance that has entered clinical studies. First, a 500-membered library of variously substituted imidazoles was prepared on a mixture of aldehyde and amine beads (Fig. 1.26). The choice of materials was based on prior knowledge of the structures of other P-glycoprotein modulators. Screening this library in whole cells led to the identification of two main leads, A, possessing an IC_{50} of 600 n*M*, and B, possessing an IC_{50} of 80 n*M*. In addition, B possessed an oral bioavailability in dogs of about 35%. These results were very encouraging.

The third stage involved making a solution-based library based on the structures of A and B. Screening produced leads C, possessing an IC_{50} of 300 n*M*, and D, with an IC_{50} of 150 n*M*. Interestingly, D was an unexpected by-product. The chemistry in libraries does not always go as intended. In addition to reasonable potency, D showed enhanced metabolic stability, so it was chosen as the lead for the next phase. Analoging around structure D lead ultimately to OC 144–093, with an IC_{50} of 50 n*M* and an

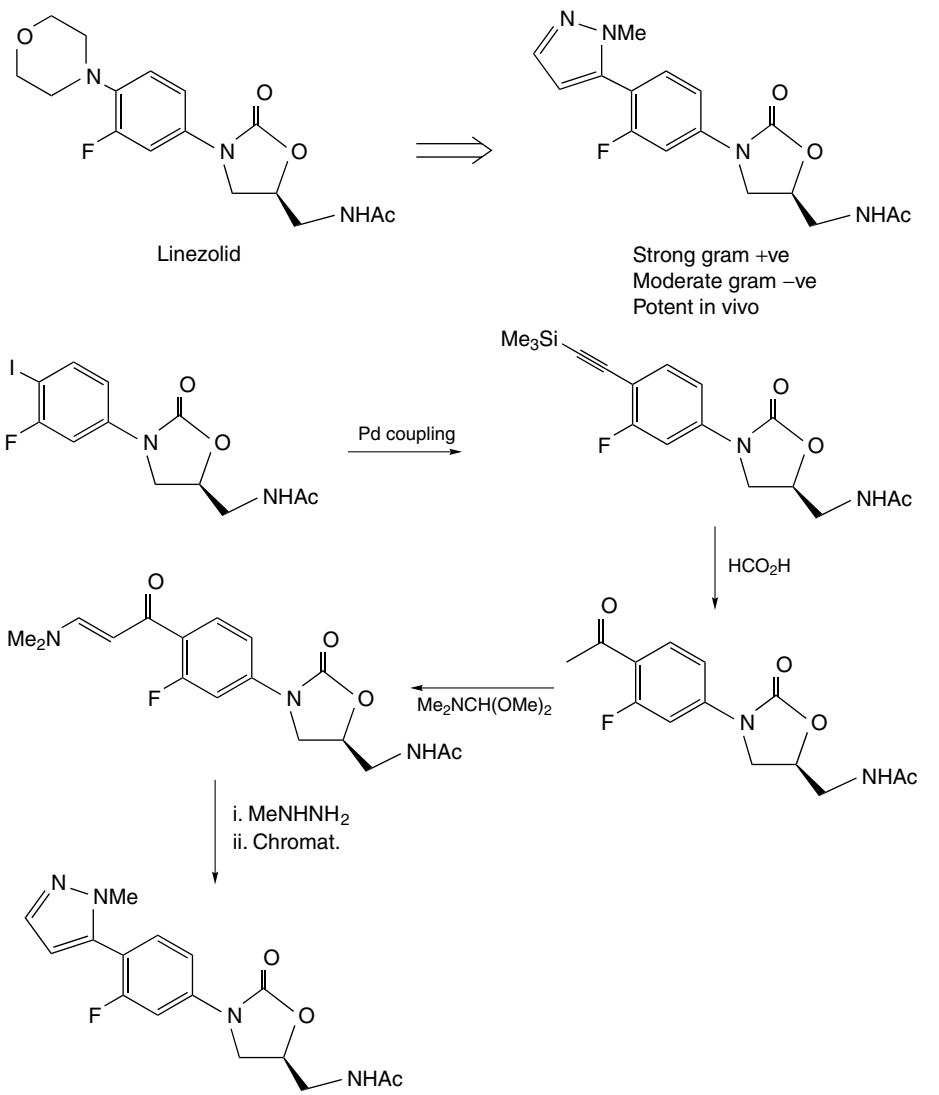


Figure 1.24. Oxazolidinone libraries.

estimated 60% bioavailability after oral administration in humans (135, 136).

Later biological studies *in vitro* and *in vivo* have shown that the agent enhances the activity of paclitaxel by interfering with its export by P-glycoprotein. It is not a substrate for CYP3A and interferes with paclitaxel metabolism only at comparatively high doses. After IV administration, OC 144–093 does not interfere with paclitaxel's pharmacokinetic profile but elevates its area under the curve when given orally. The results are interpreted as meaning that OC 144–093 interferes with gut P-glycoprotein, enhancing oral bioavailability. Further studies are in progress and it is hoped that a marketed anticancer adjunct will emerge in due course as a result of combinatorial chemistry (137).

In a different study, a search through a company compound collection was made in an attempt to find an inhibitor of the Erm family of methyltransferases. These bacterial enzymes produce resistance to the widely used macrolide-lincosaminide-streptogramin B an-

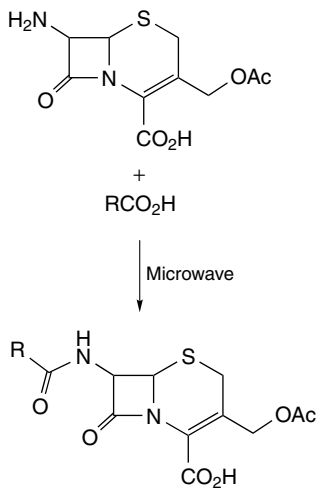


Figure 1.25. Cephalosporin libraries.

tibiotics by catalyzing *S*-adenosylmethionine–based methylation of a specific adenine residue in 23S bacterial ribosomes. This interferes with the binding of the antibiotics and conveys resistance to them. Using NMR (SAR by NMR) screening, a series of compounds including 1,3-diamino-5-thiomethyltriazine were found to bind to the active site of the enzyme, albeit weakly (1.0 m*M* for the triazine named) (Fig. 1.27). Analogs were retrieved from the collection, and analogs A, B, and C identified as promising for further work. A solution phase parallel synthesis study was performed from which compound D emerged as being significantly potent. Next a 232-compound library was prepared to discover the best R group on the left side of compound D. From this, compounds E and F emerged. These were now potent in the low micromolar range. The left side of analog E was fixed and the right side was investigated through a 411-membered library. From this, E emerged as the best substance with a K_i of 4 μM against Erm-AM and 10 μM against ErmC. Thus, starting with a very weak lead with a malleable structure, successive libraries produced analogs with quite significant potency for further exploration (138).

It is just a decade after this field became generally active, yet already most of the common drug series and hundreds of different heterocyclic classes have been prepared in library form. Originally the emphasis was on bead-based chemistry, and this actually slowed general acceptance of the method because few organic and medicinal chemists were familiar with the techniques needed to make small molecules on beads through non-iterative methods, and indeed, much of the needed technology had yet to be developed and disseminated. These problems have largely been overcome, and today the choice of beads or no beads is partly a matter of taste, the size of the libraries being made, and the length of the reaction sequences required.

The remainder of this chapter deals with selected examples that illustrates particular concepts and methodologies.

4.1 Purification

In communicating their results, chemists explicate the route with formulae and often discuss the relative strengths and weaknesses of key reagents but almost never devote time to workup. Even so, the details of the workup require attention to detail in the performance and are sometimes quite challenging. This factor becomes even more demanding in combinatorial work where the need for rapid, effective workup is intensified. Little is gained if one saves much time in construction only to have to give this back by tedious and repetitious purification schemes. Performing chemistry on beads addresses this in that simple filtration and washing often suffices. This is not as useful if the reactions do not go to completion, so considerable excess of reagents and more lengthy times are often employed to drive the reactions further to completion. Separation from solution in solid form or simple evaporation is very convenient, and manifolds for filtration and for solvent removal are commercially available. From a drugability standpoint, there is a danger in this. Compounds that separate readily from polar solvents are often of very low water solubility and present difficulties in testing. A number of commercially available combinatorial screening libraries are peppered with such substances.

Column chromatography is powerful but often labor intensive and solvent consuming. Separation of hundreds of analogs by column chromatography would be a nightmare. With smaller, focused libraries, this is often more manageable.

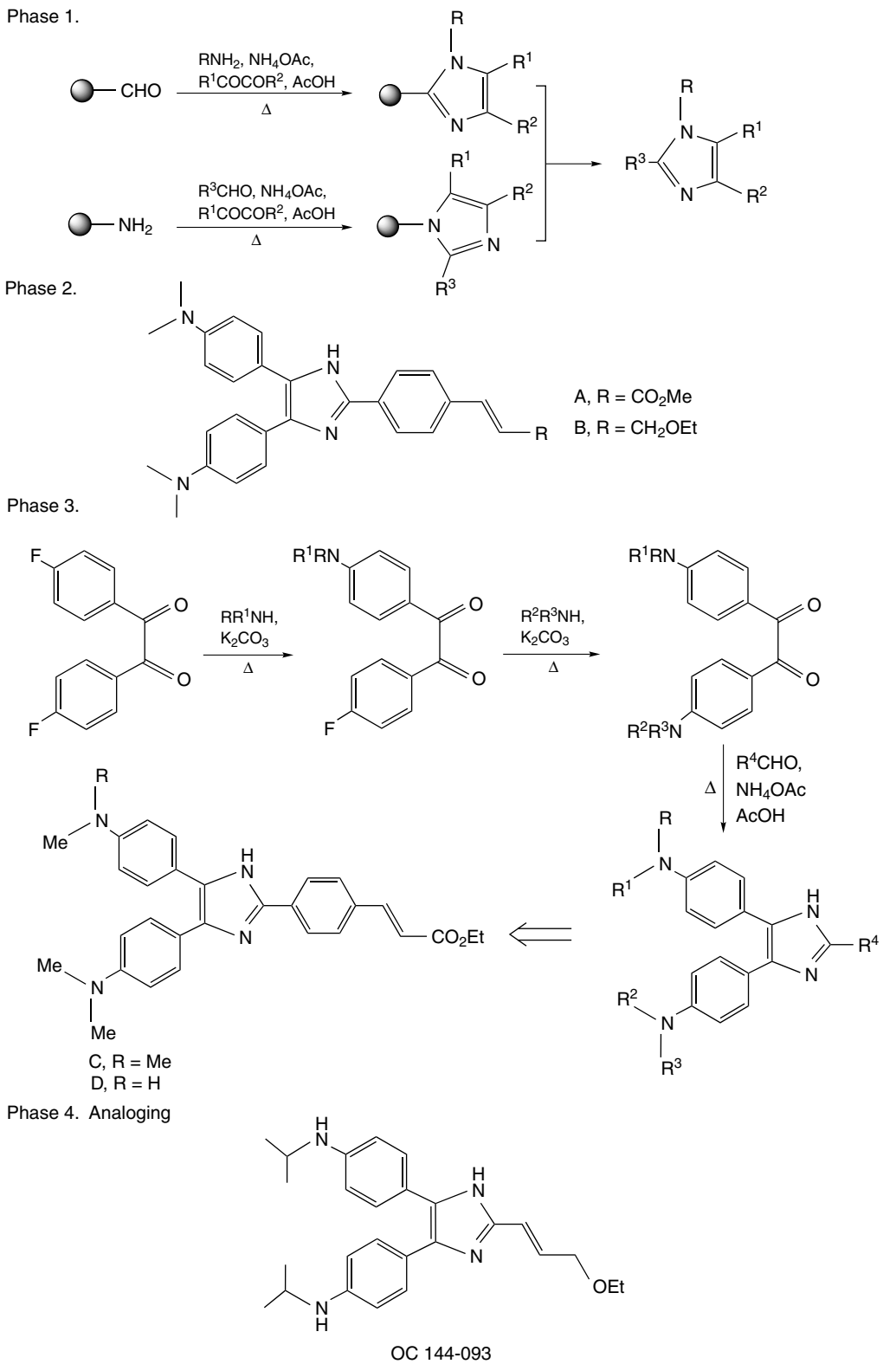
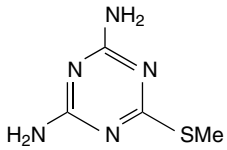


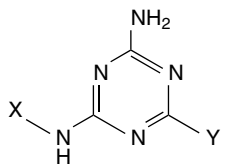
Figure 1.26. Library-based discovery of clinical candidate C 144–093.

Step 1.



K_D = 1.0 mM

Step 2. Screen in house analogs

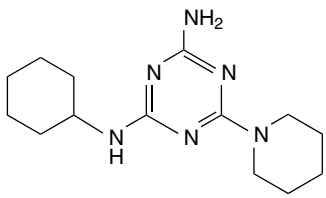


A, X = ; Y = OH K_D = 0.31 mM

B, X = H ; Y = OH K_D = 12 mM

C, X = H ; Y = N K_D = 5 mM

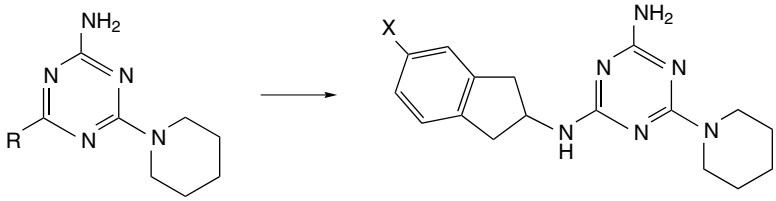
Step 3. Analoging



K_D = < 0.1 mM
K_i = 75 μM

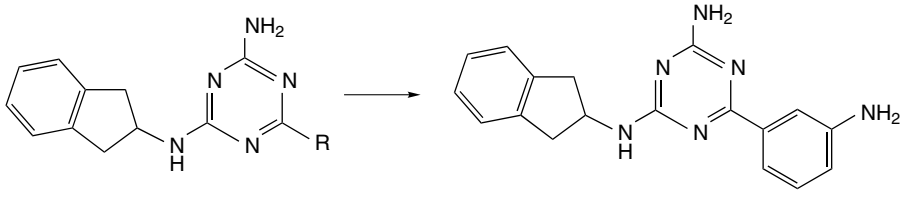
D

Step 4. Library synthesis and screening



E, X = H; K_i = 8 μM
F, X = F; K_i = 3 μM

Step 5. Second library and screening



G, K_i = 4 μM; 10 μM

Figure 1.27. A library of Erm inhibitors using SAR by NMR.

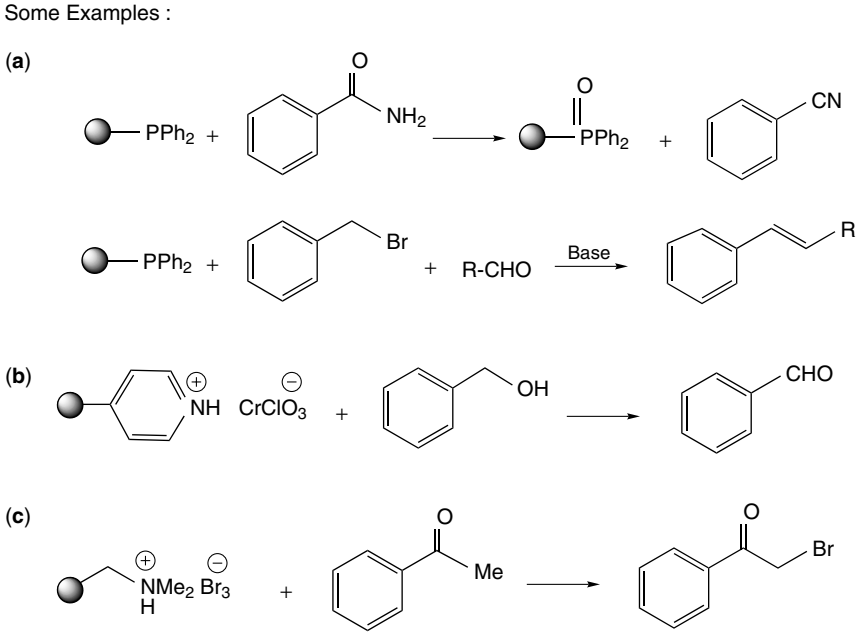


Figure 1.28. Resin tethered reagents used in combichem.

More frequently automated chromatographic reverse phase methods are employed in which round the clock separations not requiring constant human supervision are available.

Chromofiltration methods are powerful and rapid but often require study for optimization. We have found, for example, that choice of the appropriate solvent for solution-based multiple parallel synthesis can occasionally result in reaction mixtures from which the desired product can be isolated in pure form by suitable choice of absorbent and concentration or evaporation from the eluent (139). However this is generally exceptional. One can sometimes doctor silica gel, for example, to enhance its use for these purposes. After amide formation, when the acid component is used in excess, adding 1% of sodium bicarbonate to silica gel and mixing thoroughly provides a convenient way to remove reaction debris so that filtration produces pure amide on evaporation (139).

More generally applicable has been the development of many reagents tethered to solid supports. These reagents perform their intended role and then the excess reagent and the exhausted reactants can be filtered and washed away for easy product isolation. In this case, the compounds are in solution and the reagents are on the solids. A great many reagents have been prepared for use in this manner and the area has been reviewed extensively (140–142). Whereas ion exchange applications have been around a long time in the medicinal chemist's laboratory, the requirements of combinatorial chemistry have engendered a flowering of additional resins and uses. These are particularly useful in solution-based MPS but clearly find wide applications in other types of chemistry as well. An exhaustive treatment is beyond the scope of this summary, but a few examples help clarify the many uses to which this exciting technology can be put. In Fig. 1.28a, resin bound diphenylphosphine is used to reduce benzamide to benzonitrile. The diphenylphosphine oxide product is often troublesome to remove from nontethered reactions, but here is readily separated leaving the clean product behind. In Fig. 1.28b, tethered chromate cleanly oxidizes benzyl alcohol to benzaldehyde. In Fig. 1.28c, tethered bromine dimethylamino hydrobromide cleanly α-brominates acetophenone.

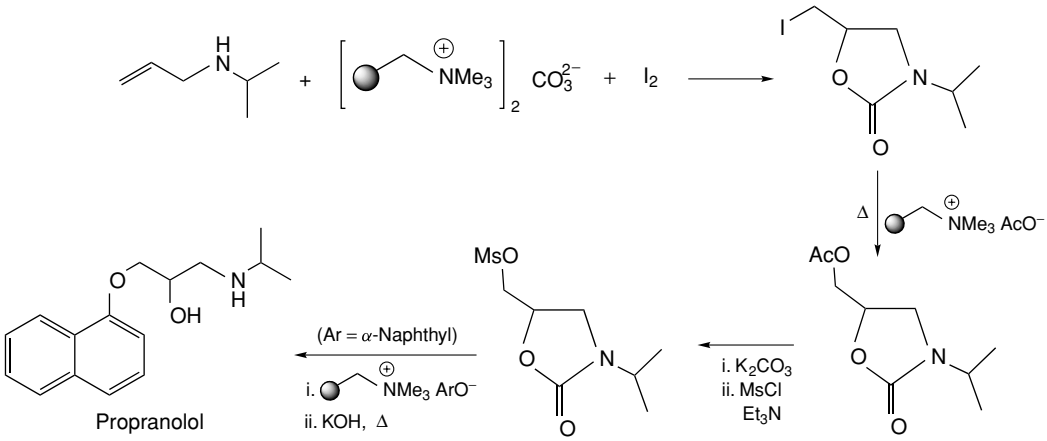


Figure 1.29. Illustration of tethered reagents in preparing propranolol.

An illustrative example of the power of this method is the resin-assisted synthesis of the adrenergic β-blocking agent, propranolol, outlined in Fig. 1.29 (143). In the six chemical steps required for this preparation, three involved the use of resin-based reagents. It is obvious that many possible variants leading to a library of related molecules could be prepared by simple modifications of the reagents and substrates.

Another important and powerful methodology employs capture or scavenger resins. Here resin-tethered bases, for example, are employed to remove acidic reaction products from reaction mixtures, and these can be regenerated for further use. Likewise, these materials can be used to remove acidic reagents or byproducts leaving the desired reaction product in the solution. Isocyanate resins are used to remove primary and secondary amines and alcohols, benzaldehyde tethered to a resin is used to remove primary amines and hydrazines, carbonate resins remove carboxylic acids and phenols, diphenylphosphine resins remove alkyl halides, and tethered trisamine removes acid chlorides, sulfonyl chlorides, and isocyanates. This methodology is similar in concept to the well-established ion exchange methodologies. The use of acid and base resins to remove ionizable products and byproducts from reaction mixtures is familiar. The use of tethered isocyanates to remove excess amine is less familiar but readily comprehended. These and analogous reagents have been widely employed and reviewed (140–142, 144). An example of the preparation of a library of drug-like molecules in solution employing resin capture methodology is illustrated in Fig. 1.30 wherein tamoxifen analogs are efficiently and cleanly prepared (145). The sequence starts with Suzuki-type coupling with a series of aryl halides. The desired intermediate is present in the product mixture with a variety of reaction detritus including diarylated material. The desired product is captured out of this stew by use of a resin bound aryl iodide which reacts exclusively with it. This device is sometimes called phase switching. The resin bound product is purified by rinsing and the reaction sequence is completed by acid release from the capture resin.

Highly fluorinated organic molecules are often insoluble at room temperature in both water and in organic solvents. At higher temperatures, however, they are soluble. Thus, heating a reaction mixture involving such a molecule to speed the reaction and then cooling on completion often allows the product to separate in pure or at least purified form by phase switching into the fluorinated solvent. This is very convenient for rapid work-up of combinatorial libraries. Recently, silica gel columns with a fluorous phase have been introduced to facilitate separations. Compounds elute from these columns in the order of their decreasing fluorine content. This can be illustrated (Fig. 1.31) by application to a library of 100 mappicine alkaloid analogs. In this case,

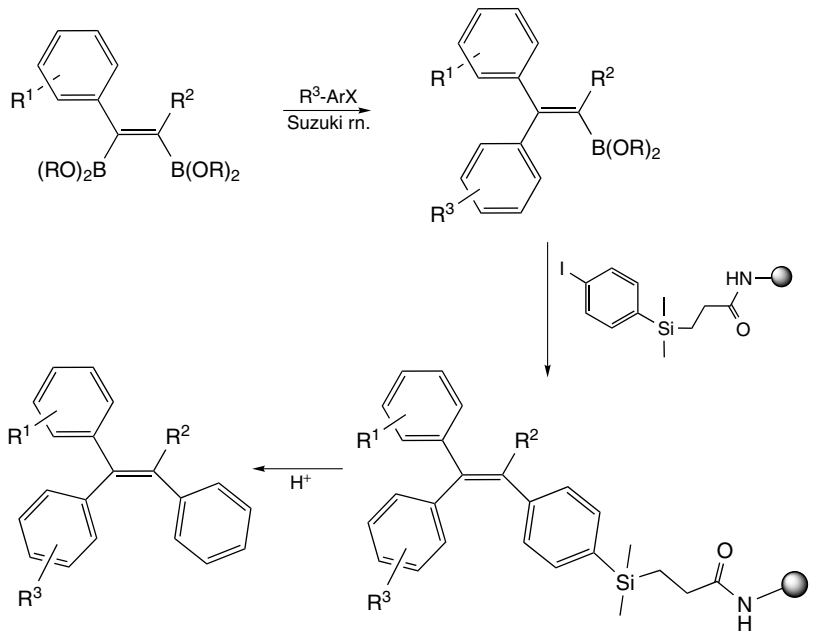


Figure 1.30. Illustration of resin capture use in a library of tamoxiphen analogs.

each analog was tagged with an arm containing one of a series of a perfluorinated hydrocarbons (CnFn+1). Syntheses could then be performed in mixtures and at the end the products were separated based on the number of fluorines in their tags. Detagging produced the individual pure products. The method is not general but is very convenient when used appropriately (146, 147).

It is also possible to remove desired reaction products, if aggressive by-products and reagents are not present, by chromatography over immobilized receptor preparations. Gel filtration is also helpful in sorting out a binding component from a mixture library containing analogs with little affinity for the pharmacological target.

Because of the impact of these newer methods, today one rarely sees a separatory funnel in a combichem laboratory. This chemistry is also comparatively "green" in that solvent needs can be greatly reduced and disposal of unwanted materials is simplified.

4.2 Synthetic Success and Product Purity

Only a relatively few census reports assessing the success rate of combinatorial library construction are available (148), but the consensus is that 85% success is fine. The level of desired purity of the components is also a matter of debate. Actionable quantitative biological data can be obtained from pure samples and uncertainties into selecting the most active constituents to pursue are increasingly introduced by assaying less pure material. Three grades of products can be distinguished. Pure usually means greater than 95% in combinatorial work. A lower but generally acceptable grade of purity is arbitrarily chosen at about 80%, and such compounds can be labeled as "practical grade." Less than this level of purity is generally unacceptable and is sometimes, disparagingly, called "practically." In very large libraries where purity analysis of each component is rarely available, inevitably one has some components in this poor state. Indeed, chemists occasionally report anecdotally finding that an active component in a library has none of the intended compound in it at all. This complicates analoging but is more satisfactory than basing SAR-based design on negative activity data wherein one can be significantly mislead in such cases.

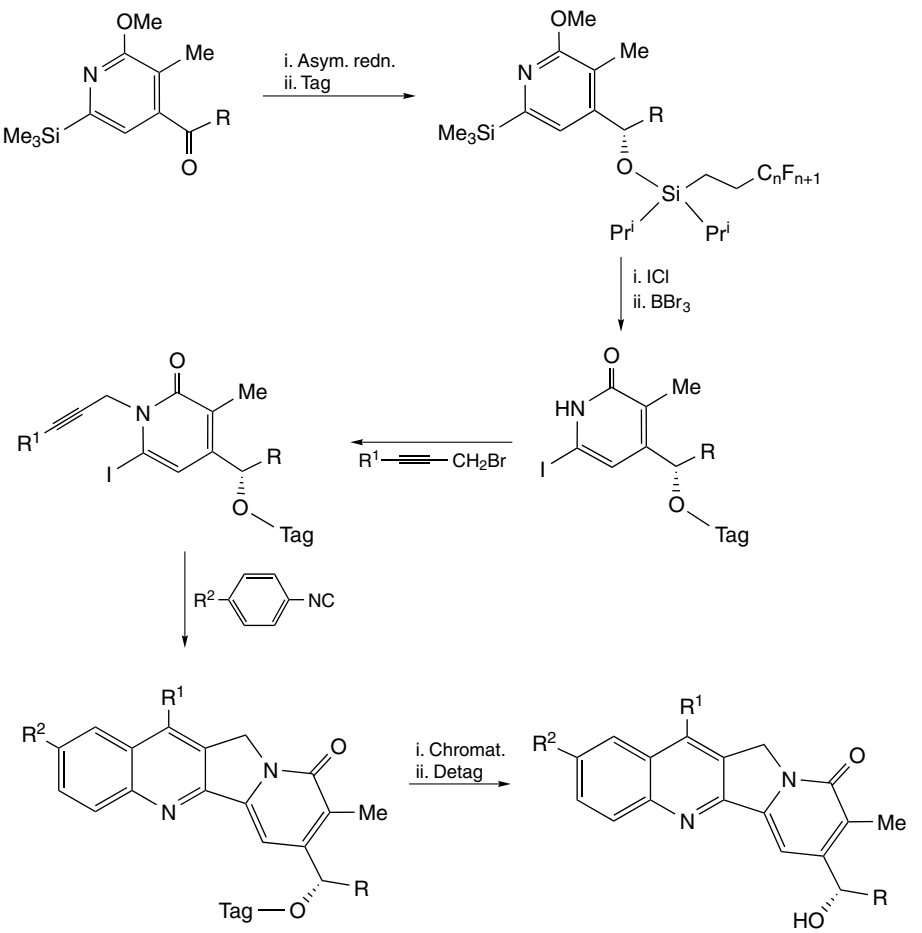


Figure 1.31. Illustration of fluorous phase methods in the synthesis of a mappacine library.

One is disturbed to note also that in a few cases where a census has been taken of very large libraries, the wrong or missing structures are not statistically distributed (149). Thus, such libraries have a structural bias. For example, the chemistry may selectively favor production of more lipophilic substances so that hydrophilic examples are underrepresented. It is hard to see how to get around this conveniently.

4.3 Resins and Solid Supports

A great variety of resins and solid supports are available for combinatorial work (151). Gel-type supports are popular and consist of a flexible polymeric matrix to which is attached functional groups capable of binding small molecules. The particular advantage of this inert support is that the whole volume of the gel is available for use rather than just the surface. Generally these consist of cross-linked polystyrene resins, cross-linked polyacrylamide resins, polyethylene glycol (PEG) grafted resins, and PEG-based resins. Surface functionalized supports have a lower loading capacity and many types are available. These include cellulose fibers, sintered polyethylene, glass, and silica gels. Composite gels are also used. These include treated Teflon membranes, kieselguhr, and the like. Brush polymers consist of polystyrene or the like grafted onto a polyethylene film or tube.

The linking functionality varies. Commonly employed resins are the Merrifield,

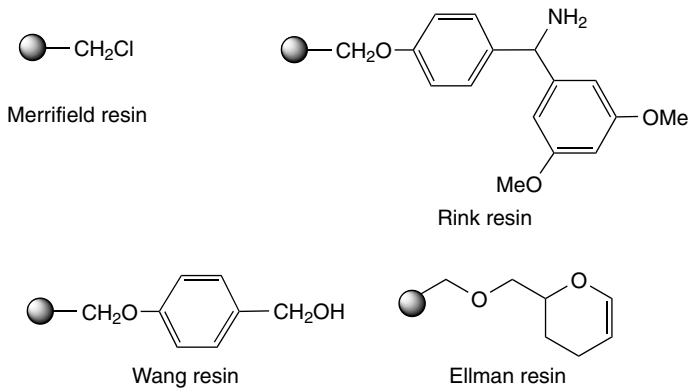


Figure 1.32. Some common resin types.

Wang, Rink, and Ellman types. These are illustrated in Fig. 1.32, and the reader can readily appreciate the kinds of chemistry that they allow.

4.4 Microwave Accelerations

Molecules possessing a permanent dipole align themselves in a microwave apparatus and oscillate as the field oscillates. This rapid motion generates intense homogeneous internal heat greatly facilitating organic reactions, especially in the solid state. For example, heat-demanding Diels-Alder reactions can take days on solid support, hours in solution, and only minutes under microwave. This has been adapted to combinatorial methods and is even compatible with a 96-well plate format (150).

4.5 Analytical Considerations

Analysis of the degree of completeness and the identity of the product is simpler than that seen in solid phase work. This closely parallels general experience in the pre-combichem days with the exception that the work load is greatly magnified. Automation is called for and hplc/tof mass spectrometry is of particular value. Even so, with very large libraries, one is usually restricted by necessity to statistical sampling and compound identification rarely goes beyond ascertaining whether the product has the correct molecular weight. If activity is found then more detailed examination takes place.

With solid-state libraries, the problem is much more complex. An enormous effort has been put into working out analysis of single beads with mass spectrometry, Raman spectroscopy, magic angle NMR, and chemiluminescence techniques being particularly popular.

4.6 Informetrics

Combinatorial synthesis and high-throughput screening generate an enormous amount of data. Keeping track of this is a job for high-speed computers. Many firms have developed their own programs for the data handling, and there are commercial packages that may be useful as well. The best of these have structure drawing capacity also.

4.7 Patents

Patent considerations are complex in combinatorial chemistry. The mass of potential data is hard to compress into a suitable format for this purpose. Commonly, patenting takes place comparatively late in a drug-seeking campaign and so differs little from traditional patenting. One notes however that the comparative speed and ease of molecule construction makes it possible to reduce to practice rather more examples that would have been possible in the one-at-a-time days.

Rather more disturbing is the increasing tendency to patent various means of making and evaluating libraries rather than focusing on their content. The fundamental purpose of patenting is to promote the useful arts and to provide protection for innovative discoveries for a period and then to share them with society in general. Patenting of means of produc-

ing libraries, if carried to an extreme, would have a dampening effect on the development of the field and so would inhibit the development of the useful arts. This should be guarded against.

5 SUMMARY AND CONCLUSIONS

Combinatorial chemistry and multiple parallel syntheses have transformed the field of medicinal chemistry for the better. The last decade has seen a revitalization and much dramatically useful technology has been discovered. No laboratory seriously involved in the search for new therapeutic agents can afford not to employ this technology.

From the vantage point of 2002, one can now look back at what has been done in the amazingly short time that this technique has been widely explored and one can see some things more clearly now and use the methodology more cunningly.

In the heady early days of combinatorial chemistry one frequently heard the opinion that existing drugs were only those to which nature or good fortune had laid a clear path. Some believed that there were large numbers of underexplored structural types that could be drugs if only they were prepared and screened. Combichem promised to make this a reality. It would be nice, indeed, if this had turned out to be true! It cannot be denied that there is some justice in this belief; speculative synthesis continues to reveal important drugs. Nonetheless, the cruel restraints that ADME and toxicity considerations place on our chemical imagination have ruined this dream of easy and unlimited progress. The present wedding of combichem with medicinal chemical knowledge is extremely powerful, and we no longer in the main waste time on collections of molecules that have no chance of becoming drugs. Clearly space for chemical diversity is larger than space for medicinal diversity.

BC (before combichem) there was little motivation for enhanced speed of synthesis. Generally, synthesis could be accomplished much more quickly than screening and evaluation of the products. Enhanced speed of construction simply produced a greater backlog of work to be done. The advent of high-throughput screening in the 1980s changed all this. The backlogs emptied rapidly, and there was a demand for more compounds. In addition, new firms were founded to take advantage of newer screening methodologies. These firms had no retained chemical libraries to screen and larger firms were reluctant to allow their libraries to be screened by outsiders. A significant part of these needs were met by the methods in this chapter. With synthesis and screening back in phase, the next choke point in the pipeline has become animal testing, pharmacokinetics, toxicity, solubility, and penetrability. These factors are presently under intensive examination in attempts to elucidate these properties in a similarly rapid fashion or to predict them so that favorable characteristics can be designed into chemical library members from the outset and thus largely avoid having to deal with them. It can readily be seen that further choke points lie distally in the pipeline and these will have to be dealt with in turn. Some time can be saved by speeding things along the way and also by dealing with the remaining constrictions in parallel rather than simultaneously, but it is difficult to see how they can all be resolved in a rapid manner. Fortunately the flow through the pipeline diminishes through increasing failure of leads to qualify for further advancement and this is helpful in reducing the magnitude of the job but the problems remaining will still be vexing. Part of the difficulty is that certain biological phenomena cannot be hurried. For example, no matter how much money and effort one is willing to throw at the problem, producing a baby requires essentially 9 months from conception. Hiring nine women will not result in producing a baby in 1 month. The problem in shortening drug seeking is further compounded in that the problem is not akin to brick laying. To produce a brick wall of a given dimensions more quickly is largely a matter of buying the bricks and hiring and motivating enough skilled labor. In drug seeking, one has to design the bricks first and develop the technology. Combichem does speed the process along but does not remove the elements of uncertainty that must be overcome. Given the strictures placed on clinical studies and their solidification in law and custom, it is

hard to see how this phase of the drug seeking sequence can be shortened through chemical effort.

High-throughput screening can be likened to hastening the process of finding a needle in a haystack. Combinatorial chemistry can be likened to the preparation of needles. Ideally one should strive to make a few more useful needles embedded in progressively smaller haystacks. This involves mating as well as is possible productive chemical characteristics with productive biological properties. Combinatorial chemistry and multiple parallel synthesis in the hands of the skillful and lucky chemist rapidly zeros in on the best combination of atoms for a given purpose. This chemist receives approbation for his/her efforts. Those who consistently come up with useless compounds will eventually be encouraged to find other work.

There has been a dramatic increase in investment in drug discovery during the last decade (estimated at 10% annually). Unfortunately this has yet to result in a burst of new introductions. Certainly chemical novelty has largely given way to potential use. Diversity no longer rules. This is perhaps the combinatorial chemist's equivalent of the businessman's mantra that whereas efficiency is doing things properly, effectiveness is doing proper things. As with much of the points being discussed, achieving a proper balance is essential.

It is interesting to note also that a 100-fold increase in screening activity has not yet resulted in a corresponding increase in the introduction of new pharmaceuticals. Part of the explanation for this is that ease of synthesis does not necessarily equate to equivalent value of the products. If each compound in chemical libraries was carefully designed and the data therefrom carefully analyzed, then the disparity would be smaller than the present experience produces. Another exculpatory factor is that much of the low hanging fruit has already been harvested and the remaining diseases are more chronic than acute and are much more complex in their etiology.

Despite all of these considerations, drug seeking is an exciting enterprise calling for the best of our talents and the appropriate use of high speed synthetic methods gives us a powerful new tool to use.

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